Is the Short Insulin Tolerance Test Safe and Reproducible?

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The short insulin tolerance test (SITT) is described as a simple method to measure insulin sensitivity. To investigate the safety and reproducibility of the SITT, 16 healthy volunteers underwent two SITTs within 1 week. Intravenous insulin (0.05 U kg^{-1} body weight) was injected into an antecubital vein. Blood samples were collected from the contralateral antecubital vein. The insulin-induced glucose disposal rate (K_{itt}) was calculated from the slope of the regression line of the logarithm of blood glucose against time during the first 3–15 min. Plasma glucose concentrations fell below 2.8 mmol l^{-1} in 4 of the 32 tests and below 2.2 mmol l^{-1} in 1 of these 4. Five subjects had mild hypoglycaemic symptoms, three of whom had plasma glucose concentrations below 2.8 mmol l^{-1} in at least one SITT. The mean K_{itt} was 4.2 % min^{-1} (range 0.8–8.4) for the first test and 3.4 % min^{-1} (range 0.1–6.8) for the second test. The mean within-subject coefficient of variation was 30.7 %. We conclude that SITT should be applied with caution especially on insulin sensitive subjects and has poor reproducibility using 0.05 U kg^{-1} body weight of insulin injection, venous sampling, uncontrolled physical activity and uncontrolled dietary composition. Whether 0.1 U kg^{-1} body weight of insulin injection and arterialized venous blood sampling as in the original description of this test can improve the reproducibility of the SITT needs further investigation. © 1998 John Wiley & Sons, Ltd.

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Introduction

Insulin resistance is important in the aetiology of conditions such as metabolic syndrome, obesity, and Type 2 diabetes mellitus. A reliable measure of insulin sensitivity for the study of the prevalence, progress of metabolic disease or effectiveness of specific therapies is necessary. Three methods have been widely employed to measure insulin sensitivity *in vivo*: the glucose clamp technique,[1] the modified insulin suppression test,[2] and the minimal model method.[3] However, all require sophisticated equipment and trained personnel and are expensive to perform. Using fasting insulin levels as a substitute for the measurement of insulin resistance is simple but can be used only in subjects with normal glucose tolerance.[4]

The short insulin tolerance test (SITT) has been used to assess insulin sensitivity but it has two potential disadvantages. There is at least a theoretical risk of hypoglycaemia and the result can potentially be affected by counterregulatory responses.[5] Bonora *et al.*[6] demonstrated that counterregulatory hormone concentrations remain at basal levels during the first 15 min and concluded that the glucose disposal rate in the first 15 min of the SITT represents whole body insulin-mediated glucose uptake. However, there remains the possibility that the simpler methods may be associated with less precision than more complex protocols. We set out to determine the safety and reproducibility of SITT.

Patients and Methods

Sixteen healthy volunteers (7 men, 9 women; mean age 41 (range 26–59) years) were recruited. Their mean body mass index (BMI) was 22.9 (range 19.1 to 29.8) kg m^{-2} (Table 1). They had no family history of diabetes, normal fasting blood glucose, liver and renal function. All subjects gave written informed consent before taking part in the study.

The tests were performed between 08:00 and 09:30 and after a 10 h overnight fast. Each subject underwent two SITTs (first test and second test) with an interval of not more than 1 week. The antecubital vein was cannulated for blood sampling and a fasting sample taken for glucose and insulin assay (first study only). A bolus of Human Actrapid (Novo Nordisk, Denmark) 0.05 U kg^{-1} body weight was injected into the contralateral antecubital vein. Blood sampling was carried out at 0, 1, 3, 5, 7, 9, 11, 13, and 15 min after the injection of insulin. Symptoms and signs of hypoglycaemia were recorded. The test was terminated by injection of glucose.

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Table 1. Baseline characteristics of the 16 subjects

Subject	Sex	Age (yr)	BMI (kg m^{-2})
1	M	36	22.1
2	M	34	22.8
3	M	31	22.0
4	M	24	22.8
5	M	33	19.1
6	M	26	26.7
7	M	59	26.8
8	F	46	21.6
9	F	47	20.7
10	F	36	29.8
11	F	45	23.3
12	F	52	19.7
13	F	49	20.7
14	F	54	19.9
15	F	43	25.8
16	F	44	23.3

BMI, body mass index.

Plasma glucose was determined at the bedside using a glucose oxidase method (Astra-8, Beckman, CA, USA). The inter-assay coefficient of variation (CV) of glucose was 3.7 % at 5.2 mmol l^{-1}. The intra-assay CV of glucose was 1.2 % at 4.7 mmol l^{-1}. Serum insulin was assayed using a commercial RIA kit (Diagnostic Products Corp., Los Angeles, CA, USA). The samples were collected in unheparinized tubes, centrifuged at room temperature and stored at –70°C for later analysis. All of the samples from each subject were analyzed with the same assay. The inter-assay CV of insulin was 8.7 % at 34.5 pmol l^{-1}. The intra-assay CV of insulin was 3.5 % at 86.7 pmol l^{-1}.

This study was approved by the Human Research Committee of the China Medical College Hospital.

Statistical Analysis

Linear regression was used to estimate the slope of the decline in log transformed blood glucose concentration. The slope was multiplied by –100 to derive the glucose disposal rate (K_{itt}). The K_{itt} was equivalent to the percentage decline in blood glucose per min calculated by the formula of $69.3/t_{1,2}$, where $t_{1,2}$ was the time that blood glucose fell from a certain value at 3 min to half that value. Variability was represented by the CV. The CV was calculated as the SD/mean × 100, where SD = standard deviation. The correlation was analyzed by Pearson's correlation test. Values of $p < 0.05$ were considered as statistically significant.

Results

Seventeen subjects underwent the first short insulin tolerance test. Sixteen subjects received the second test within an interval of 1 week. One subject fainted after removal of the cannula (blood glucose 3.6 mmol l^{-1} on a home monitoring meter from Medisense Inc., Waltham MA, USA) during the first test and did not participate in the second test. The mean plasma glucose at 3 min for the first test was 5.1 (range 4.7–5.6) mmol l^{-1} and for the second test was 5.2 (range 4.5–5.7) mmol l^{-1}. The mean plasma glucose at 15 min was 3.2 (range 1.8–4.7) mmol l^{-1} for the first test and 3.6 (range 2.4–5.3) mmol l^{-1} for the second test. Plasma glucose concentrations fell below 2.8 mmol l^{-1} in 4 of the 32 tests and below 2.2 mmol l^{-1} in 1 of these 4. The plasma glucose concentrations of subject 13 fell to 1.8 mmol l^{-1} and 2.4 mmol l^{-1} in the separated tests. Five subjects reported mild hypoglycaemic symptoms (dizziness, palpitation, hungry sensation), three of these had plasma glucose concentrations below 2.8 mmol l^{-1} in at least one SITT. The mean K_{itt} was 4.2 (range 0.8–8.4) % min^{-1} for the first test and 3.4 (range 0.1–6.8) % min^{-1} for the second test. The mean within-subject CV was 30.7 (range 2.0–110) % (Table 2). The mean fasting serum insulin was 71.0 (range 20.0–264.5) pmol l^{-1} (Table 2). There was a positive correlation between fasting serum insulin levels and the K_{itt} of the first SITT ($r = –0.8461$, $p < 0.001$).

Discussion

SITT is a simple method to measure insulin sensitivity. However, it carries the possibility of hypoglycaemia. Young *et al.*[7] showed that shortening SITT from 30 min to 15 min did not always prevent significant hypoglycaemia in healthy insulin sensitive subjects even when the insulin dosage was reduced from 0.1 U kg^{-1} body weight to 0.05 U kg^{-1} body weight. The plasma glucose concentration of one of their subjects approached 2.5 mmol l^{-1} as soon as 11 min after insulin injection. They also found comparable degrees of hypoglycaemia in other subjects. Five of our subjects developed mild hypoglycaemic

Table 2. The fasting insulin, glucose disposal rate (K_{itt}) after insulin injection, and within-subject CV of the 16 subjects

Subject	Fasting insulin (pmol l^{-1})	K_{itt} (1st test)	K_{itt} (2nd test)	Absolute difference	CV (%)
1	61.3	4.8	4.1	0.7	9.1
2	135.4	2.3	3.9	1.6	36.5
3[a]	53.5	4.4	2.2	2.2	47.1
4	29.9	4.2	3.2	1.0	19.1
5[a]	75.5	2.7	5.0	2.3	42.3
6[a]	20.0	4.5	3.3	1.2	21.8
7	57.0	3.3	3.7	0.4	8.1
8	86.7	3.9	2.3	1.6	36.5
9	60.8	4.0	2.8	1.2	24.5
10	80.4	3.8	3.2	0.6	12.1
11	45.0	3.5	4.4	0.9	16.1
12	37.9	3.5	3.4	0.1	2.0
13[a]	27.4	8.4	6.8	1.6	14.8
14[a]	34.9	6.8	2.2	4.6	72.3
15	264.5	0.8	0.1	0.7	110.0
16	66.2	5.7	4.4	1.3	18.2
mean	71.0	4.2	3.4	1.4	30.7

[a]Hypoglycaemic symptoms developed in these subjects.

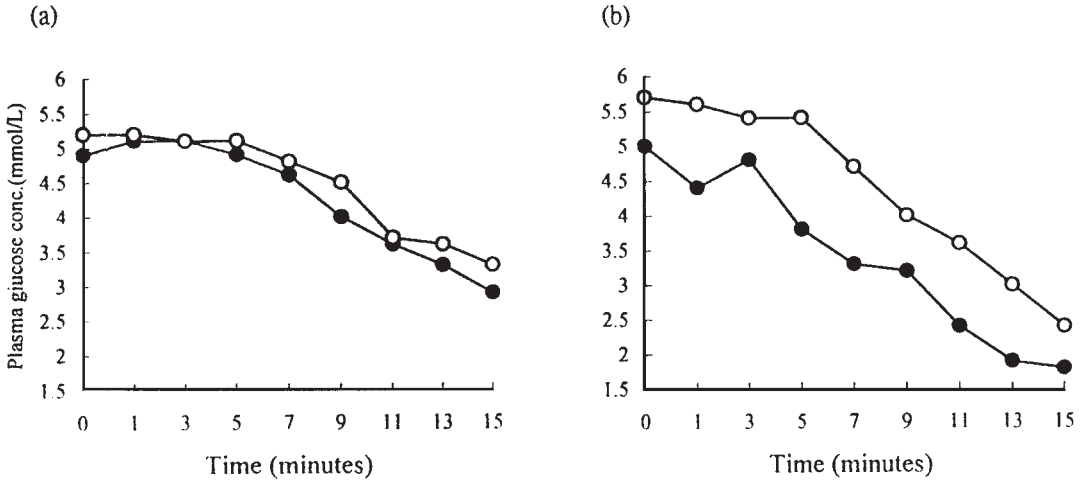

Figure 1. The glucose excursion curves of two subjects in the first SITT (●) and the second SITT (○). (a) Subject 1, no hypoglycaemic symptoms: the plasma glucose at 15 min was 2.9 mmol l^{-1} (1st SITT) and 3.3 mmol l^{-1} (2nd SITT), respectively. (b) Subject 13, with hypoglycaemic symptoms: the plasma glucose at 15 min was 1.8 mmol l^{-1} (1st SITT) and 2.4 mmol l^{-1} (2nd SITT), respectively

symptoms, three of whom had plasma glucose concentrations below 2.8 mmol l^{-1} in at least one SITT, and plasma glucose concentrations fell below 2.8 mmol l^{-1} in an additional test (in one case to less than 2.2 mmol l^{-1}), Figure 1. Only we and Young *et al.*[7] have reported hypoglycaemia in SITT, despite several other reports of the use of the test.[6–10] Ethnic differences in insulin sensitivity[11–13] may explain the similarities between our data and those of Young *et al.*,[7] in which the subjects were mostly Hong Kong residents of Chinese descent (our subjects were all Chinese). In any event, the potential for dangerous hypoglycaemia in insulin sensitive subjects indicates that the SITT needs adequate supervision. For safety reasons, it may be advisable to terminate the SITT at 11 min and the validity of such a shortened test needs to be investigated. This may be less of a problem in the study of insulin resistant subjects, such as diabetic patients.

We used venous blood and an insulin bolus of 0.05 U kg^{-1} body weight instead of the arterialized venous blood and the insulin dose of 0.1 U kg^{-1} body weight described in earlier papers, in order to minimise risk of hypoglycaemia. Young *et al.*[7] found that a reduction in the dose did not significantly alter the rate of hypoglycaemia but did increase the CV of the test (from 9.4 % to 15 %). Using arterialized venous blood, Akinmokun *et al.*[8] found a CV of 6 % using an insulin dose of 0.1 U kg^{-1} body weight, while with a 0.05 U kg^{-1} body weight insulin dose, Hirst *et al.*[9] had a CV of 13 % and Gelding *et al.*[10] of 6.9 ± 2.6 %; Akinmokun *et al.*[8] showed there was no correlation between euglycemic clamp and SITT using venous blood sampling, despite good correlation with arterialized samples. Arterialization of venous sampling improved the reproducibility of the first phase insulin response to intravenous glucose tolerance test[14] and it is likely that the reduction in insulin dose and failure to arterialize venous blood contributed to the high CV of the SITT of our study.

We did not measure counterregulatory hormones in our study but five subjects experienced mild hypoglycaemic symptoms. If we exclude these subjects and that of the one subject with a highly variable response, the mean CV of our study becomes 18.2 %, close to that of Young *et al.*[7]

Insulin sensitivity is affected by various daily events such as an alteration of dietary composition and exercise. Chen *et al.*[15] showed that when dietary carbohydrate was increased from 30 % to 41 % of daily caloric intake, the insulin sensitivity index rose in young men, as did glucose disappearance rate in elderly men when carbohydrate intake increased from moderate (49 %) to very high (85 %). Lampman *et al.*[16] also showed that *in vivo* insulin sensitivity improved following exercise training. A failure to control the physical activity and dietary composition of our volunteers before testing may also contributes to the high variance of our study.

In conclusion, because SITT can induce frank hypoglycaemia, caution must be exercised when it is used in insulin sensitive subjects. The test has poor reproducibility when using 0.05 U kg^{-1} body weight of insulin, venous sampling, and uncontrolled physical activity and diet beforehand. Further investigation is needed to determine whether 0.1 U kg^{-1} body weight of insulin injection and arterialized venous blood sampling can improve the reproducibility of the SITT and is safe.

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